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14. ABSTRACT: The BCR-ABL tyrosine kinase inhibitor imatinib mesylate (Gleevec) is the preferred treatment for human chronic myeloid leukemia (CML), but does not cure mice with BCR-ABL-induced acute lymphoblastic leukemia (ALL), similar to CML lymphoid blast crisis. The inability of imatinib to cure CML in mice leads us to hypothesize that a BCR-ABL kinase activity-independent pathway also plays a critical role in the development of this disease. We identified Src kinases as key molecules in this BCR-ABL kinase activity-independent pathway, and they are essential for leukemic cells to survive imatinib treatment and for CML transition to lymphoid blast crisis. Inhibition of both SRC and BCR-ABL kinase activities affords complete B-ALL remission. However, leukemic stem cell pathways must be targeted for curative therapy of Ph+ leukemia. We have identified CML stem cells in mice, and found that these cells are insensitive to imatinib therapy. Our study suggests that Src kinases may be effective in inhibiting leukemic stem cells, and combination therapy using a BCR-ABL/Src inhibitor and an anti-stem cells agent would be beneficial to CML patients. Our work will provide a new therapeutic strategy for CML.

15. SUBJECT TERMS
CML, BCR-ABL ONCOGENE, PH+ LEUKEMIA, MOLECULAR TARGETS, SIGNALING PATHWAY, MOUSE MODEL

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Introduction

Human chronic myelogenous leukemia (CML) is induced by the BCR-ABL oncogene. CML often initiates in a chronic phase and eventually progresses to an advanced terminal blastic phase, in which either acute myeloid or acute lymphoid leukemia (AML or ALL) develops. Thus, a successful treatment of CML requires dealing with diseases in both chronic and advanced or blastic phases. BCR-ABL is an oncogenic kinase. It is generally believed that shutting down the kinase activity of BCR-ABL will completely inhibit its functions, leading to inactivation of its downstream signaling pathways, consequently stopping cellular transformation by BCR-ABL. The BCR-ABL tyrosine kinase inhibitor imatinib mesylate (STI571 or Gleevec) is the preferred treatment for chronic phase CML patients [1]. However, imatinib was unable to abrogate BCR-ABL-expressing leukemic cells [2], and induced cellular and clinical drug resistance [3-9]. Moreover, imatinib is much less effective in treating CML blastic phase patients [10,11], and primitive leukemia cells are insensitive to imatinib treatment [12]. We and others have shown that imatinib prolongs survival of mice with BCR-ABL-induced CML [13,14], but does not cure the disease [13]. We have also shown that imatinib does not cure mice with BCR-ABL-induced ALL, similar to CML lymphoid blast crisis [13]. Because imatinib is a strong inhibitor against BCR-ABL kinase activity, the inability of imatinib to cure CML and ALL in mice [13] leads us to hypothesize that a BCR-ABL kinase activity-<u>independent</u> pathway also plays a critical role in the development of CML. Our preliminary data suggest that In this application, we will test our hypotheses using mouse models of human CML [15-17] and ALL [15,18]. Specifically, we will study the role of this kinase-independent pathway in the development of chronic phase CML and advanced phase CML represented by B-ALL, and test if targeting this pathway is therapeutically effective.

Body

With the support partially from this grant, we made significant progress in the projects outlined in the Statement of Work. Below, I describe in detail what we have accomplished so far. I need to point out that due to the availabilities of reagents to be used at the time of experiments, the original proposed timelines for individual projects were changed accordingly, with some projects completed in time, early or late. The results for all completed experiments have published (Hu Y, Swerdlow S, Duffy TM, Weinmann R, Lee FY, Li S. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph^+ leukemia. **Proc Natl Acad Sci USA** 103(45):16870-16875, 2006), and <u>corresponding numbers of figures in this paper are used below</u>.

Task 1. To determine the effect of Lyn, Hck, and Fgr in survival and self-renewal of BCR-ABL-expressing hematopoietic stem cells (HSCs):

a. Develop a FACS analysis to identify BCR-ABL-expressing HSCs.

By FACS analysis, we had successfully identified BCR-ABL-expressing hematopoietic stem cells (HSCs) as CML stem cells in mice. These stem cells are $Lin^-Sca\text{-}1^+c\text{-}Kit^+$ (Fig. 6B).

b. Determine the effect of Lyn, Hck, and Fgr in survival and self-renewal of BCR-ABL-expressing HSCs using Src knockout mice.

This experiment requires large numbers of $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ mice. Due to the shortage of these mice for unexpected reasons, we will have to do this experiment in the second year. Now we are close to having sufficient numbers of the mice for use.

c. Further demonstrate the role of Src kinases in survival and self-renewal of BCR-ABL-expressing HSCs using the dual Src/BCR-ABL kinase inhibitor BMS-354825.

This experiment is to provide further support for the genetic determination of the effect of Lyn, Hck, and Fgr in survival and self-renewal of BCR-ABL-expressing HSCs using Src knockout mice (described in Task 1b above). Because the project described in Task 1b was delayed due to the availability of the $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ mice, we will also carry out this experiment in the second year.

Task 2. To test whether lack of Lyn, Hck, and Fgr prevents transition of CML chronic phase to lymphoid blast crisis:

a. Test whether Src kinases play a role in CML transition to lymphoid blast crisis using Src knockout mice in a serial transplantation assay.

We tested whether Src kinases play a role in CML transition to lymphoid blast crisis using a serial transplantation assay [16]. Mice were transplanted with BCR-ABL-transduced bone marrow (BM) cells from either wild type or $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ mice to induce CML, and BM cells from the CML mice were transferred into lethally irradiated syngeneic recipient mice. Mice receiving wild type CML BM cells developed ALL diagnosed by FACS using $GFP^{+}CD43^{+}B220^{+}CD19^{+}$ as markers, whereas mice receiving $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ CML BM cells did not develop this disease (Fig. 3E). These results demonstrate that lack of Lyn, Hck, and Fgr prevents transition of CML chronic phase to lymphoid blast crisis.

b. Further test whether Src kinases play a role in CML transition to lymphoid blast crisis using BMS-354825 in a serial transplantation assay.

We will perform this experiment in the second year.

Task 3. To determine whether activation of Src kinases by BCR-ABL provides a mechanism for insensitivity of ALL in CML blastic phase to imatinib treatment:

a. Demonstrate the role of Src kinases in causing the insensitivity of ALL to imatinib using wild type BCR-ABL and BMS-354825.

We treated ALL mice with BMS-354825, which inhibits both BCR-ABL and Src kinase activity, or with imatinib, which only inhibits BCR-ABL kinase activity. We found that BMS-354825 more markedly prolongs survival of the mice than imatinib did (Fig. 3A). The therapeutic effects of these two drugs correlated with reduced levels of GFP^{+} leukemic cells in peripheral blood of the treated mice (Fig. 3B). To demonstrate that the weaker therapeutic effect of imatinib is not due to a failure of imatinib to inhibit BCR-ABL kinase activity *in vivo*, we examined if imatinib significantly inhibits BCR-ABL phosphorylation in pre-B leukemic cells from peripheral blood of the treated mice. We found that imatinib and BMS-354825 similarly inhibited BCR-ABL phosphorylation in mice (Fig. 3C). These results demonstrate that Src kinases are responsible for causing the insensitivity of ALL to imatinib.

b. Further demonstrate the role of Src kinases in causing the insensitivity of ALL to imatinib using the BCR-ABL-T315I mutant and BMS-354825.

We treated mice with BCR-ABL-T315I-induced ALL with BMS354825 or imatinib to compare survival of the two treatment groups of mice, allowing only investigating the effect of Src inhibition on ALL, because BCR-ABL-T315I mutant is no longer sensitive to inhibition of its kinases activity by imatinib and BMS-354825 but its activation of Src kinases is not affected and can be inhibited by BMS-435825. We found that inhibition of Src kinases by BMS-435825 significantly prolonged survival of B-ALL mice, but imatinib did not (Fig. 2D), demonstrating that the ineffectiveness of imatinib in treating ALL mice was due to the Src kinase activation that was not inhibited by imatinib.

Key Research Accomplishments

1. Establishment of a critical role of Src kinases in the development of advanced CML;

2. Identification of CML stem cells in mice.

Reportable Outcomes

Some results have been recently published in the journal of PNAS (Hu Y, Swerdlow S, Duffy TM, Weinmann R, Lee FY, Li S. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph^+ leukemia. **Proc Natl Acad Sci USA** 103(45):16870-16875, 2006)

Conclusions

Src kinases are essential for leukemic cells to survive imatinib treatment and for CML transition to lymphoid blast crisis. Src kinases are valuable therapeutic targets, and inhibition of both Src and BCR-ABL kinase activities by BMS-354825 is critical to ALL treatment.

References

1. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001;344:1031-1037.
2. Marley SB, Deininger MW, Davidson RJ, Goldman JM, Gordon MY. The tyrosine kinase inhibitor STI571, like interferon-alpha, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. Exp Hematol. 2000;28:551-557.
3. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001;293:876-880.
4. Weisberg E, Griffin JD. Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood. 2000;95:3498-3505.
5. le Coutre P, Tassi E, Varella-Garcia M, et al. Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood. 2000;95:1758-1766.
6. Mahon FX, Deininger MW, Schultheis B, et al. Selection and characterization of *BCR-ABL* positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. Blood. 2000;96:1070-1079.

7. Shah NP, Nicoll JM, Nagar B, et al. Multiple *BCR-ABL* kinase domain mutants confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crise chronic myeloid leukemia. Cancer Cell. 2002;2:117-125.
8. Branford S, Rudzki Z, Walsh S, et al. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood. 2002;99:3472-3475.
9. von Bubnoff N, Schneller F, Peschel C, Duyster J. BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. Lancet. 2002;359:487-491.
10. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med. 2001;344:1038-1042.
11. Talpaz M, Sawyers CL, Kantarjain H, et al. Activity of an ABL specific tyrosine kinase inhibitor in patients with BCR/ABL positive acute leukemias, including chronic myelogenous leukemia in blast crisis. Oncologist. 2000;5:282-283 (Abstr.).
12. Holtz MS, Slovak ML, Zhang F, Sawyers CL, Forman SJ, Bhatia R. Imatinib mesylate (ST1571) inhibits growth of primitive malignant progenitors in chronic myelogenous leukemia through reversal of abnormally increased proliferation. Blood. 2002;99:3792-3800.
13. Hu Y, Liu Y, Pelletier S, et al. Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. Nat Genet. 2004;36:453-461.
14. Wolff NC, Ilaria RL, Jr. Establishment of a murine model for therapy-treated chronic myelogenous leukemia using the tyrosine kinase inhibitor STI571. Blood. 2001;98:2808-2816.
15. Li S, Ilaria RL, Jr., Million RP, Daley GQ, Van Etten RA. The P190, P210, and p230 forms of the *BCR/ABL* oncogene induce a similar chronic myeloid leukemia-like syndrome in mice but have different lymphoid leukemogenic activity. J Exp Med. 1999;189:1399-1412.
16. Pear WS, Miller JP, Xu L, et al. Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. Blood. 1998;92:3780-3792.
17. Zhang X, Ren R. Bcr-Abl efficiently induces a myeloproliferative disease and production of excess interleukin-3 and granulocyte-macrophage colony-stimulating factor in mice: a novel model for chronic myelogenous leukemia. Blood. 1998;92:3829-3840.
18. Roumiantsev S, de Aos IE, Varticovski L, Ilaria RL, Van Etten RA. The src homology 2 domain of Bcr/Abl is required for efficient induction of chronic myeloid leukemia-like disease in mice but not for lymphoid leukemogenesis or activation of phosphatidylinositol 3-kinase. Blood. 2001;97:4-13.

Appendices

Hu Y, Swerdlow S, Duffy TM, Weinmann R, Lee FY, Li S. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph^+ leukemia. **Proc Natl Acad Sci USA** 103(45):16870-16875, 2006.

Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph + leukemia in mice

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Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph$^+$ leukemia in mice

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It is generally believed that shutting down the kinase activity of BCR-ABL by imatinib will completely inhibit its functions, leading to inactivation of its downstream signaling pathways and cure of the disease. Imatinib is highly effective at treating human Philadelphia chromosome-positive (Ph$^+$) chronic myeloid leukemia (CML) in chronic phase but not Ph$^+$ B cell acute lymphoblastic leukemia (B-ALL) and CML blast crisis. We find that SRC kinases activated by BCR-ABL remain fully active in imatinib-treated mouse leukemic cells, suggesting that imatinib does not inactivate all BCR-ABL-activated signaling pathways. This SRC pathway is essential for leukemic cells to survive imatinib treatment and for CML transition to lymphoid blast crisis. Inhibition of both SRC and BCR-ABL kinase activities by dasatinib affords complete B-ALL remission. However, curing B-ALL and CML mice requires killing leukemic stem cells insensitive to both imatinib and dasatinib. Besides BCR-ABL and SRC kinases, stem cell pathways must be targeted for curative therapy of Ph$^+$ leukemia.

dasatinib | imatinib | SRC kinases

The human Philadelphia chromosome, Ph, arises from a translocation between chromosomes 9 and 22 and results in formation of the chimeric and constitutively activated BCR-ABL tyrosine kinase. Philadelphia chromosome-positive (Ph$^+$) leukemias induced by the BCR-ABL oncogene include chronic myeloid leukemia (CML) and B cell acute lymphoblastic leukemia (B-ALL). CML often initiates in a chronic phase and eventually progresses to a terminal blastic phase in which either acute myeloid or acute B-lymphoid leukemia develops. Some Ph$^+$ leukemia patients, however, have B-ALL as their first clinical appearance. It is generally believed that shutting down the kinase activity of BCR-ABL will completely inhibit its functions, leading to inactivation of its downstream signaling pathways. Therefore, current therapeutic efforts have focused on targeting BCR-ABL kinase activity by using kinase inhibitors.

The BCR-ABL tyrosine kinase inhibitor imatinib mesylate (also known as Gleevec) is the standard of care for Ph$^+$ leukemia. Imatinib induces a complete hematologic response in chronic-phase CML patients (1). However, imatinib does not completely eliminate BCR-ABL-expressing leukemic cells (2, 3), and patients frequently present with drug resistance (4). Imatinib prolongs survival of mice with BCR-ABL-induced CML (5, 6) but does not cure the disease (5). Recently, three BCR-ABL kinase inhibitors, dasatinib (7), AP23464 (8), and AMN107 (9), have been shown to inhibit almost all imatinib-resistant BCR-ABL mutants; the exception is the T315I mutant, which is present in 15–20% of imatinib-resistant patients. Dasatinib also is a potent inhibitor of SRC family kinases, but the role of the anti-SRC activity of this compound in Ph$^+$ leukemia therapy has not been studied (7). For unknown reasons, imatinib is much less effective in treating CML blastic-phase patients and patients with Ph$^+$ B-ALL (10), which has not been shown to be related to the BCR-ABL kinase domain mutations, the most common type of imatinib resistance. Because imatinib is a strong inhibitor of BCR-ABL kinase activity, the inability of imatinib to cure CML and B-ALL in mice (5) suggests that inactivation of BCR-ABL kinase activity alone is insufficient to control the disease.

We have previously shown that the three SRC-family kinases, LYN, HCK, and FGR, are activated by BCR-ABL in lymphoid leukemic cells and are required for the development of B-ALL (5). Furthermore, cells from patients resistant to imatinib expressed an activated form of LYN (11). We reasoned that inhibition of BCR-ABL kinase activity by imatinib might not inactivate SRC kinases activated by BCR-ABL in lymphoid leukemic cells, and this may explain the relatively poor activity of imatinib against Ph$^+$ B-ALL and lymphoid blast crisis. In this study, we provide evidence that imatinib does not inactivate the SRC signaling pathway activated by BCR-ABL and that this pathway is essential for the development of Ph$^+$ B-ALL. We also show that other targets need to be identified to inhibit imatinib-insensitive leukemic stem cells for Ph$^+$ B-ALL and CML.

Results

SRC Kinases Remain Active After Imatinib Inhibition of BCR-ABL Kinase Activity. We tested the hypothesis that imatinib may not inactivate SRC kinases activated by BCR-ABL using a BCR-ABL-expressing pre-B cell line (ENU) (5). The cells were treated with or without imatinib. Compared with cells bearing the empty vector, Western blot analysis showed that SRC kinases were activated in cells expressing one of two forms of BCR-ABL (P190 and P210, which differ in molecular weight and are expressed predominantly in Ph$^+$ ALL and CML, respectively), and imatinib treatment markedly inhibited BCR-ABL kinase activity but did not result in a decrease in SRC activation (Fig. 1*A*). These results indicate that, although imatinib was very effective in inhibiting BCR-ABL phosphorylation, it was unable to affect BCR-ABL-stimulated phosphorylation of SRC kinases. To demonstrate this finding further, we used the P190 or P210 form of BCR-ABL to transform mouse bone marrow (BM) cells. These cells were then treated with imatinib. Imatinib inhibited BCR-ABL phosphorylation, resulting in decreased phosphorylation of downstream signaling molecule CrkL, but did not affect BCR-ABL-stimulated phosphorylation of SRC kinases (Fig. 1*B*). These observations indicate that, in imatinib-treated BCR-ABL-expressing cells, SRC kinases are still active and may participate in cellular transformation by BCR-ABL.

Author contributions: S.L. designed research; Y.H., S.S., and S.L. performed research; R.W. and F.Y.L. contributed new reagents/analytic tools; Y.H., T.M.D., and S.L. analyzed data; and S.L. wrote the paper.

The authors declare no conflict of interest.

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Abbreviations: B-ALL, B cell acute lymphoblastic leukemia; BM, bone marrow; CML, chronic myeloid leukemia; HSC, hematopoietic stem cell.

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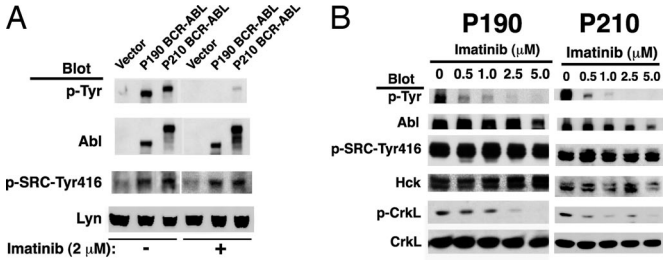

Fig. 1. SRC kinases remain active after inhibition of BCR-ABL kinase activity by imatinib. (*A*) Activation of SRC kinases by BCR-ABL does not require BCR-ABL kinase activity. P190- or P210-expressing ENU cells were cultured in the presence or absence of imatinib for 12 h, and ENU cells bearing empty vector were used as controls. Protein lysates were analyzed by Western blotting with antibodies against phosphotyrosine (*p*-Tyr), ABL, activated SRC kinases (*p*-SRC-Tyr-416) (29), and LYN. (*B*) BCR-ABL-transduced BM cells were cultured under Whitlock–Witte conditions for 5 days. The cells were treated with imatinib at the concentrations indicated for 2 days. Protein lysates were analyzed by Western blotting with the antibodies indicated.

Key Role of SRC Kinases in Malignant Transformation of B-Lymphoid Cells. To investigate the role of SRC kinase activation in transformation of B-lymphoid cells and in the survival and proliferation of leukemic cells, we showed that v-SRC, an active form of SRC kinase, directly transformed B-lymphoid cells *in vitro* (Fig. 2*A*), suggesting that activated SRC alone is sufficient to stimulate aberrant proliferation of hematopoietic precursors. To examine whether inhibition of SRC kinases attenuates transformation of mouse BM cells by BCR-ABL, we used a dual SRC/BCR-ABL inhibitor dasatinib (Fig. 2*B*). We transformed mouse BM cells with the BCR-ABL-T315I mutant that is resistant to inhibition of BCR-ABL kinase activity by both imatinib (4, 12, 13) and dasatinib (7). This allowed us to dissociate the inhibitory effects on the BCR-ABL kinase vs. the effects on SRC kinases. Dasatinib reduced survival (Fig. 2*C*) and induced apoptosis (data not shown) of the leukemic cells, demonstrating that BCR-ABL-activated SRC kinases in imatinib-treated cells play a critical role in BCR-ABL-mediated transformation of B-lymphoid cells.

We further investigated the role of SRC kinase in B-ALL development using a mouse model of B-ALL (14). We treated mice with B-ALL induced by BCR-ABL-T315I with imatinib or dasatinib. Imatinib showed no therapeutic effect, whereas dasatinib significantly prolonged survival of the mice ($P < 0.01$) (Fig. 2*D*). Dasatinib inhibited SRC kinase activity *in vivo* (Fig. 2*E*). These results indicate that SRC kinases play a critical role in B-ALL development. However, targeting SRC kinases alone did not cure the disease (Fig. 2*D*), which may be due to the incomplete inhibition of SRC kinase activity *in vivo* at the dose of dasatinib used (Fig. 2*E*); a higher dose of dasatinib may further improve survival of the mice. To further support the role of SRC kinases in B-ALL development, we compared growth potential of BCR-ABL-transduced wild-type and $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ BM cells. We monitored the levels of pre-B leukemic cells expressing BCR-ABL (represented by GFP expression) over a 4-week time period in peripheral blood of mice receiving BCR-ABL-transduced wild-type or $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ BM cells. Levels of GFP^+B220^+ B-lymphoid leukemic cells were significantly lower in mice receiving BCR-ABL-transduced $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ BM cells than in those receiving BCR-ABL-transduced wild-type BM cells at all time points measured, although there was an initial increase in leukemic cells in mice receiving the transduced $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ BM cells (Fig. 2*F*). Strikingly, in mice receiving the transduced $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ BM cells, leukemic cells almost disappeared 5 weeks following B-ALL induction, whereas in the mice receiving the transduced wild-type BM cells, ≈45% of leukemic cells persisted (Fig. 2*G*).

Inhibition Solely of BCR-ABL Kinase Activity Without SRC Kinase Inhibition Is Insufficient for B-ALL Treatment. Because SRC kinases are still active when BCR-ABL phosphorylation is inhibited by

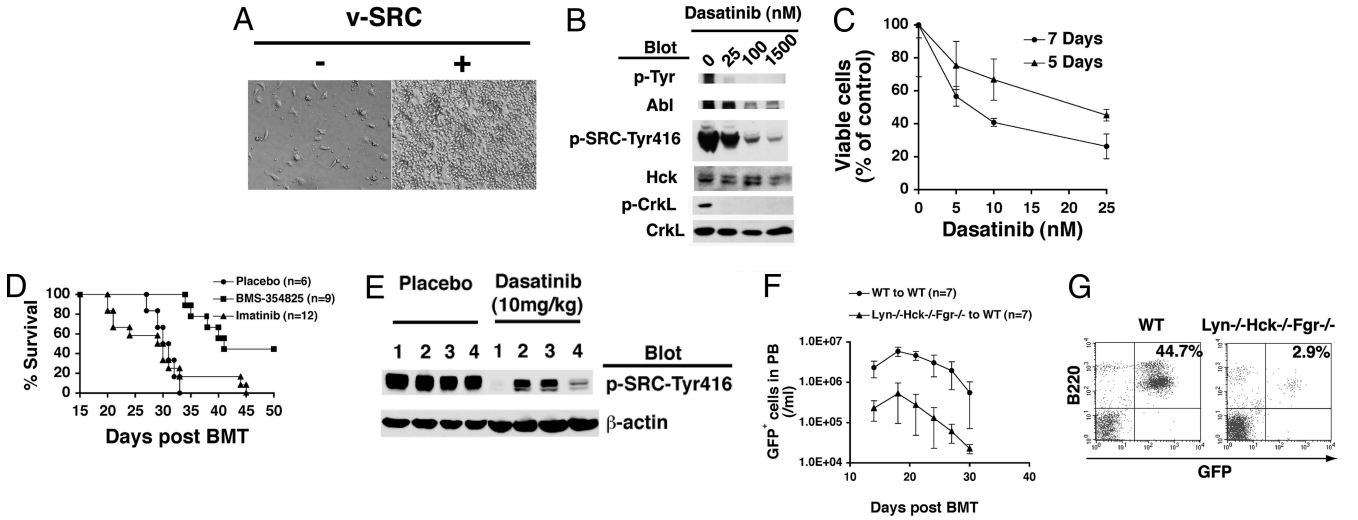

Fig. 2. SRC kinases play a critical role in maintaining survival and promoting proliferation of pre-B leukemic cells. (*A*) BM cells from B6 mice were transduced with the empty vector or v-SRC retrovirus and cultured under Whitlock–Witte conditions for 14 days. (*B*) Dasatinib inhibits activity of both BCR-ABL and SRC kinases. BCR-ABL-transduced BM cells were cultured under Whitlock–Witte conditions for 5 days. Different concentrations of dasatinib were added to the culture for 48 h, and protein lysates were analyzed by Western blotting. (*C*) Inhibition of SRC kinases reduces survival of BCR-ABL-T315I-expressing B-lymphoid cells. BCR-ABL-T315I-transduced BM cells were cultured at 1×10^5 cells per well in 24-well plates, and different concentrations of dasatinib were added to the culture for 5 or 7 days. Viable cells were counted. (*D*) Therapeutic effect of imatinib and dasatinib on BCR-ABL-T315I-induced B-ALL. BMT, BM transplantation. (*E*) *In vivo* inhibition of SRC kinase activity with dasatinib. Mice with BCR-ABL-T315I-induced B-ALL were treated with a placebo or dasatinib for 3 days. After the last dose, leukemic cells from peripheral blood of the mice were analyzed by Western blotting. Each lane represents a mouse from the indicated treatment group. (*F*) BCR-ABL-transduced wild-type or Lyn/Hck/Fgr triple knockout BM cells were transplanted into wild-type recipient mice to induce B-ALL. GFP^+ cell counts (percentage of GFP^+ cells × white blood cell count) were measured at different time points after the induction of leukemia. (*G*) Percentages of GFP^+ B-leukemic cells in peripheral blood were determined by FACS analysis as described in *F*.

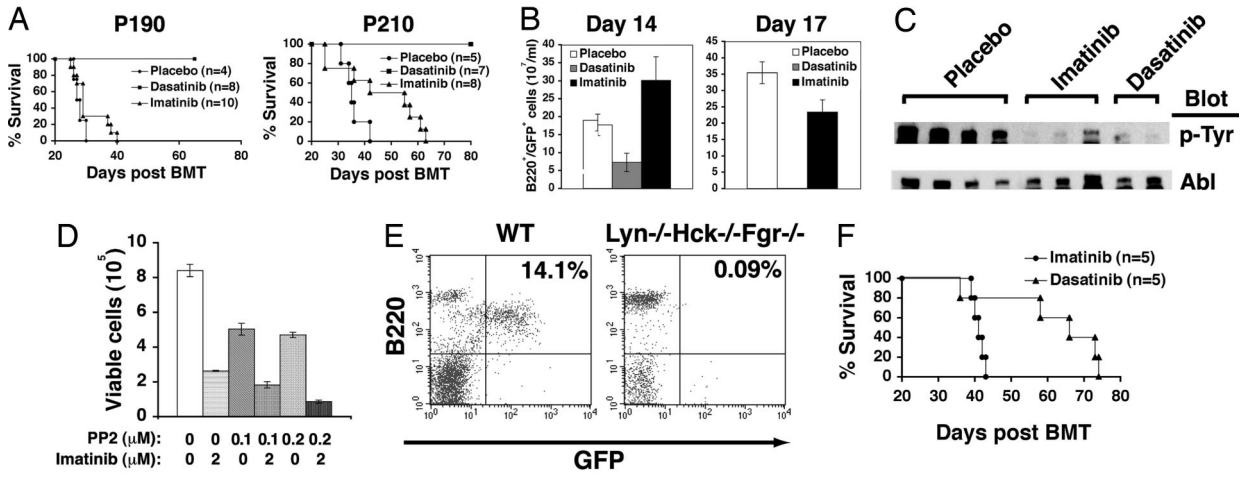

Fig. 3. Simultaneous targeting of kinase activity of both BCR-ABL and SRC kinases results in long-term survival of mice with B-ALL. (*A*) Mice with BCR-ABL-induced B-ALL were treated with a placebo, imatinib, or dasatinib. BMT, BM transplantation. (*B*) Reduction of GFP^+ leukemic cells in peripheral blood of the treated B-ALL mice. (*C*) *In vivo* inhibition of BCR-ABL autophosphorylation by imatinib and dasatinib. B-ALL mice were treated with placebo, imatinib, or dasatinib for 3 days. After the last dose, leukemic cells from the pleural effusion were analyzed by Western blotting. Each lane represents a mouse from the indicated treatment group. (*D*) The SRC-selective kinase inhibitor PP2 alone or with imatinib has an inhibitory effect on proliferation of BCR-ABL-transduced BM cells in Whitlock–Witte culture. The transduced cells were cultured at 1×10^5 per well in 24-well plates for 5 days, and the two drugs were added to the culture for the last 2 days. Viable cells were counted. (*E*) Lack of LYN, HCK, and FGR prevents CML transition to lymphoid blast crisis. Wild-type and Lyn/Hck/Fgr triple knockout BM cells from CML mice were transferred into wild-type recipient mice to assay CML transition to B-ALL by FACS analysis of GFP^+ B-leukemic cells in peripheral blood. (*F*) Dasatinib, but not imatinib, is effective at suppressing p53-deficient leukemic cells in B-ALL mice.

imatinib (Fig. 1), we examined whether inhibition of BCR-ABL kinase activity alone, with SRC kinase still active, is sufficient to control B-ALL. We treated mice with BCR-ABL-induced B-ALL with imatinib (which inhibits only BCR-ABL kinase activity) or with dasatinib (which inhibits both BCR-ABL and SRC kinase activity). Imatinib had a weak therapeutic effect on B-ALL (Fig. 3*A*), suggesting that inhibition solely of BCR-ABL kinase activity is insufficient to control the disease. By contrast, dasatinib maintained long-term survival of the mice with B-ALL induced by P190 or P210 BCR-ABL (Fig. 3*A*), indicating that both BCR-ABL kinase activity and SRC pathway must be targeted for treating this disease. The therapeutic effects of these two drugs on B-ALL correlated with reduced levels of GFP^+ leukemic cells in peripheral blood of the treated B-ALL mice (Fig. 3*B*). The weak therapeutic effect of imatinib (Fig. 3*A*) cannot be attributed to an inability to inhibit BCR-ABL kinase activity *in vivo*, because imatinib significantly inhibited BCR-ABL phosphorylation to a similar extent compared with dasatinib in leukemic cells from pleural effusion of the treated B-ALL mice (Fig. 3*C*). To exclude the possibility that the better therapeutic effect of dasatinib over imatinib (Fig. 3*A*) could be attributed to the potency difference of these drugs on BCR-ABL kinase activity (7) but not to the additional anti-SRC effect of dasatinib (Fig. 2*B*), we treated BCR-ABL-transformed BM cells under Whitlock–Witte conditions with a SRC kinase inhibitor PP2 alone (15) (which did not inhibit BCR-ABL kinase activity at the concentrations used in this study), with imatinib alone, and with both PP2 and imatinib. Either drug alone inhibited proliferation of the cells, but both drugs together had a much stronger inhibitory effect (Fig. 3*D*). These results support the critical role of SRC kinases in B-ALL development.

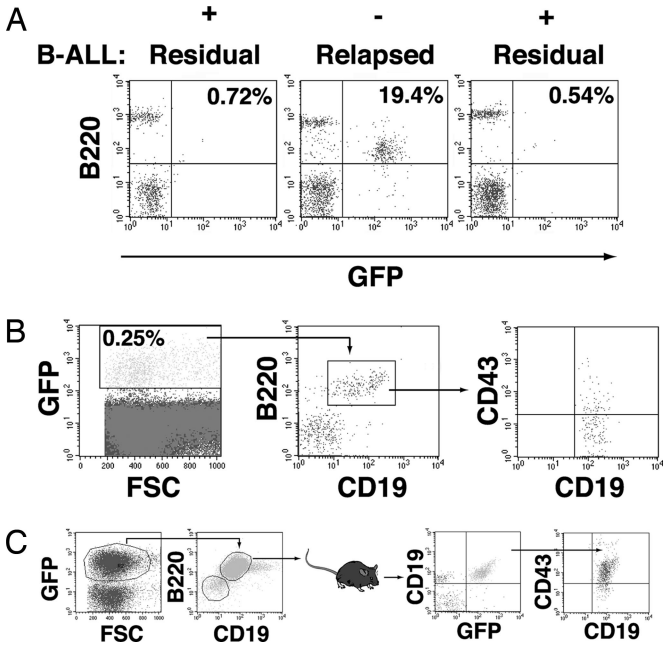

Fig. 4. Dasatinib efficiently kills highly proliferating B-leukemic cells, but not stem cells, in B-ALL mice. (*A*) B-ALL reappeared in most of the mice after dasatinib treatment stopped (−); the relapsed mice remained sensitive to dasatinib therapy (+). (*B*) A low level of GFP^+ pro- or pre-B cells (<1%) persisted in dasatinib-treated mice. (*C*) $B220^+/CD43^+$ pro-B leukemic cells function as leukemic stem cells in B-ALL. The sorted $GFP^+/B220^+/CD19^+$ cells from BM of B-ALL mice transfer B-ALL to secondary recipients after 2 months, and leukemic cells in peripheral blood are $B220^+/CD43^+$ pro-B cells.

Progression to Lymphoid Blast Crisis CML Requires Activation of SRC Kinases. Chronic-phase CML advances to blastic phase. We genetically tested whether SRC kinases play a role in CML transition to lymphoid blast crisis using a serial transplantation assay (16). Mice were transplanted with BCR-ABL-transduced BM cells from either wild-type or $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ mice to induce CML, and BM cells from the CML mice were subsequently transferred into recipient mice. Mice receiving wild-type CML BM cells developed B-ALL, shown by $GFP^+/B220^+$ leukemic cells in peripheral blood, whereas none of the mice receiving $Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$ CML BM cells developed this disease (Fig. 3*E*). These results indicate that CML transition to

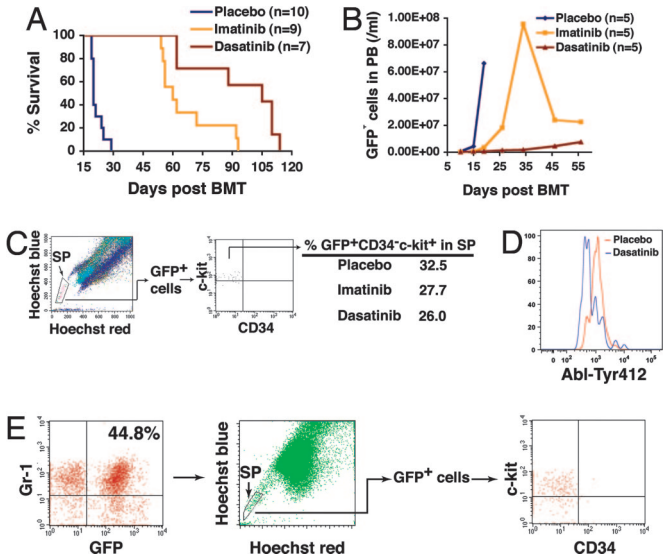

Fig. 5. Imatinib and dasatinib fail to eradicate BCR-ABL-expressing HSCs completely. (*A*) CML mice treated with imatinib and dasatinib. BMT, BM transplantation. (*B*) GFP$^+$ leukemic cell counts in peripheral blood (PB) of CML mice treated with imatinib and dasatinib. (*C*) Comparison of the percentages of BCR-ABL-expressing HSCs (GFP$^+$CD34$^-$c-*kit*$^+$Hoe$^-$) in side populations (SP) of BM cells from placebo-, imatinib-, and dasatinib-treated CML mice. (*D*) Dasatinib inhibits BCR-ABL kinase activity in CML stem cells. BM cells from CML mice were treated with a placebo or dasatinib (100 nM) in culture for 24 h, and BCR-ABL-expressing HSCs (GFP$^+$CD34$^-$c-*kit*$^+$Hoe$^-$) were identified by FACS. Intracellular levels of BCR-ABL phosphorylation were determined by FACS with anti-Abl-Y412 antibody, which detects the active form of BCR-ABL. (*E*) A representative CML mouse treated with dasatinib for 16 weeks still contains large numbers of BCR-ABL-expressing HSCs.

lymphoid blast crisis requires SRC kinases. CML progression is associated with additional genetic changes, including mutations in the tumor suppressor genes INK4[a], pRB, and p53 (17–19). A recent study showed that Arf gene loss enhances the oncogenicity of imatinib and limits imatinib response to BCR-ABL-induced B-ALL in mice (20). To test whether SRC kinases are effective targets for B-ALL when tumor suppressor gene function is defective, BCR-ABL-transduced BM cells from p53-deficient mice were transplanted into lethally irradiated wild-type recipient mice followed by treatment with imatinib or dasatinib. Dasatinib was more effective than imatinib in suppressing p53-deficient pre-B leukemic cells, but these mice eventually died (Fig. 3*F*). These results suggest that loss of p53 function causes reduction of dasatinib response to BCR-ABL-induced B-ALL, although a significant degree of response remained.

Pro-B Leukemic Cells Are Identified as B-ALL Stem Cells, and Continuous Treatment with Dasatinib May Prevent Them from Developing into B-ALL. We tested whether dasatinib could completely eradicate leukemic cells in B-ALL mice, leading to cures. Although dasatinib remarkably prolonged survival of B-ALL mice (Fig. 3*A*), a small percentage of GFP$^+$ cells (<1%) remained in the peripheral blood of these mice even after 3 months of treatment (Fig. 4*A*). After treatment was stopped, B-ALL reappeared in most mice (Fig. 4*A*) within 1 month for P190BCR-ABL-induced and within 2 months for P210BCR-ABL-induced B-ALL. The relapsed B-ALL mice were treated again with dasatinib, and the percentage of GFP$^+$ cells dropped again to <1% (Fig. 4*A*) and remained at this level during continuous drug treatment for 2 months (data not shown). After two rounds of treatment discontinuations, relapses, and retreatment, the mice remained sensitive to the next round of dasatinib therapy (data not shown). Still, a low level (<1%) of GFP$^+$ cells persisted in the BM of the treated B-ALL mice, and these cells were capable of transferring the same disease to secondary recipient mice (data not shown). These results indicate that continuous administration of dasatinib could prevent these residual cells from developing into fatal B-ALL, although this compound did not completely kill the residual leukemic cells (Fig. 4*A*).

We identified the cell types of these residual GFP$^+$ cells as B220$^+$/CD43$^+$ and B220$^+$/CD43$^-$ pro-/pre-B cells (Fig. 4*B*), and these progenitor leukemic cells may have acquired self-renewal capacity and function as B-ALL stem cells. To test this hypothesis, we sorted by FACS the B220$^+$/CD19$^+$/GFP$^+$ cells from the BM of B-ALL mice, followed by transplantation of the cells into recipient mice. These mice developed B-ALL after 2 months, and leukemic cells in peripheral blood were CD19$^+$/CD43$^+$ pro-B cells (Fig. 4*C*). We conclude that CD19$^+$/B220$^+$/CD43$^+$ pro-B cells expressing BCR-ABL can function as B-ALL stem cells. To support this conclusion, we transferred purified BCR-ABL-expressing CD19$^+$/B220$^+$/CD43$^+$ pro-B cells into recipient mice; these cells induced leukemia and had potential to differentiate (data not shown).

Dasatinib Significantly Prolongs Survival of CML Mice but Does Not Eradicate CML Stem Cells. Dasatinib is very effective in controlling B-ALL (Fig. 3*A*). We tested whether dasatinib also is effective at treating CML in mice. CML mice treated with dasatinib lived significantly longer than those treated with imatinib (Fig. 5*A*), which correlated with significantly lower numbers of BCR-ABL-expressing leukemic cells in peripheral blood (Fig. 5*B*) compared with placebo- or imatinib-treated mice. However, all dasatinib-treated CML mice eventually died of this disease (Fig. 5*A*), indicating that, like imatinib (21), this drug may not eradicate leukemic stem cells in CML mice. Because CML in mice originates from multilineage repopulating cells (14), we tested whether dasatinib kills BCR-ABL-expressing hematopoietic stem cells (HSCs) *in vivo*. We used BALB/c mice to induce CML because we used this strain in our therapeutic experiments in this study (Figs. 3*A* and 5*A*). We treated CML mice with a placebo, imatinib, or dasatinib for 14 days, starting from day 8 after CML induction by BCR-ABL, and found that BCR-ABL-expressing HSCs (GFP$^+$CD34$^-$c-*Kit*$^+$Hoe$^-$) existed in the side population (22) of BM cells from the imatinib- or dasatinib-treated CML mice (Fig. 5*C*). This observation indicates that neither imatinib nor dasatinib completely eradicates BCR-ABL-expressing HSCs, suggesting that neither drug will cure CML and that targeting at least one additional component of BCR-ABL-expressing HSCs is required for curing the disease. Because analysis of HSCs in side populations for the existence of the stem cells is not quantitative, we further analyzed BCR-ABL-expressing HSCs in dasatinib-treated CML mice (in B6 background) by identifying the GFP$^+$Lin$^-$c-*kit*$^+$Sca-1$^+$ population. Compared with placebo-treated mice, dasatinib reduced the numbers of BCR-ABL-expressing HSCs but failed to eradicate these cells completely in CML mice (data not shown), consistent with the finding using BALB/c mice (Fig. 5*C*). This biological effect on BCR-ABL-expressing HSCs does not support the possibility that inability of dasatinib to completely eradicate BCR-ABL-expressing HSCs may be attributed to the failure of dasatinib to access the stem cells, because we detected inhibition of intracellular BCR-ABL phosphorylation by dasatinib in the stem cells (Fig. 5*D*). The inability of dasatinib to cure CML mice is not attributed to the appearance of BCR-ABL-T315I clone in the mice, because CML mice treated with dasatinib for ≈3 months contained >40% of GFP$^+$Gr-1$^+$ cells, among which there were large numbers of stem cells (Fig. 5*E*), and sequencing analysis of isolated genomic DNA from BM cells

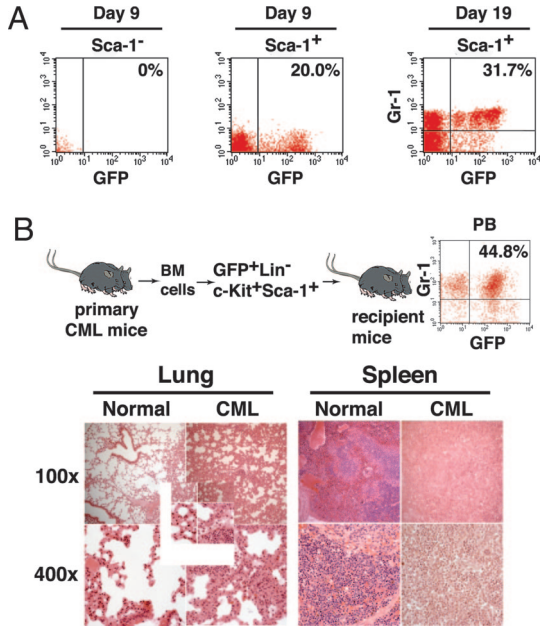

Fig. 6. Identification of BM cell populations that function as CML stem cells. (*A*) BCR-ABL-transduced BM cells from B6 mice were sorted by Sca-1 MACS columns (Miltenyi Biotec, Gladbach, Germany), followed by transferring a Sca-1$^-$ or Sca-1$^+$ population into B6 mice (1×10^5 cells per mouse; four mice per cell population group) to induce CML. GFP$^+$ myeloid cells (Gr-1$^+$) in peripheral blood (PB) of the mice were examined at days 9 and 19 after the induction of leukemia. All mice receiving the Sca-1$^+$ population died of CML by day 42. (*B*) BCR-ABL-expressing HSCs function as CML stem cells. BM cells from CML mice in B6 background were sorted by FACS for BCR-ABL-expressing HSCs (GFP$^+$Lin$^-$c-*kit*$^+$Sca-1$^+$), followed by transfer into lethally irradiated B6 mice (2×10^4 cells per mouse). GFP$^+$ myeloid cells (Gr-1$^+$) were detected in peripheral blood. In contrast to the normal control mice, CML mice showed complete infiltration of the lungs with myeloid leukemic cells and complete disruption of follicular architecture of the spleen by infiltrating leukemic cells.

of these mice did not show T315I mutation in the BCR-ABL kinase domain (data not shown). The failure of imatinib to eradicate BCR-ABL-expressing HSCs is not related to c-*kit* function, because both imatinib and dasatinib inhibit c-*kit* (23). These results suggest that inhibition of BCR-ABL kinase activity alone is insufficient to eradicate CML stem cells.

To identify CML stem cells, we tested whether BCR-ABL-expressing HSCs function as the stem cells. We first sorted C57BL/6 (B6) BM cells transduced with BCR-ABL retrovirus into two separate populations: Sca-1$^-$ and Sca-1$^+$. These two populations of cells were transferred, respectively, into B6 mice. Only the mice receiving BCR-ABL-transduced Sca-1$^+$ cells developed and died of CML, diagnosed by detecting GFP$^+$ myeloid cells (Gr-1$^+$) in the peripheral blood of the mice (Fig. 6*A*). This result suggests that early BM progenitors contain CML stem cells. To narrow down the specific cell lineages that function as CML stem cells, HSCs (Lin$^-$c-*kit*$^+$Sca-1$^+$) were sorted out from BM cells transduced with BCR-ABL retrovirus, followed by transfer into recipient mice. The mice developed and died of CML (data not shown). To confirm definitively that BCR-ABL-expressing HSCs are CML stem cells, we isolated BM cells from primary CML mice and sorted out the BCR-ABL-expressing HSCs (GFP$^+$Lin$^-$c-*Kit*$^+$Sca-1$^+$) by FACS. The sorted cells were transferred into recipient mice, and the mice developed and died of CML (Fig. 6*B*), indicating that BCR-ABL expressing HSCs function as CML stem cells.

Discussion

Our findings provide evidence that inhibition solely of BCR-ABL kinase activity is effective at treating Ph$^+$ B-ALL and CML in mice but is not sufficient to achieve complete control of these two types of leukemia. This failure is partially caused by BCR-ABL activation of signaling pathways, such as SRC kinases, that are not inhibited by imatinib and essential to leukemia development. Sustained activation of these pathways would allow leukemic cells to survive treatment with compounds that inhibit only BCR-ABL kinase activity until the emergence of drug resistance. Simultaneous targeting of these pathways and BCR-ABL kinase activity would provide a vastly improved therapeutic approach to chemotherapy of Ph$^+$ leukemias. This strategy is in contrast to a general idea that complete and sole inhibition of BCR-ABL kinase activity would completely inhibit BCR-ABL functions.

SRC kinases play a critical role in the development of BCR-ABL-induced B-ALL (5). Sole inhibition of BCR-ABL kinase activity with kinase inhibitors will not shut down the SRC pathway, suggesting the existence of a BCR-ABL kinase activity-independent pathway. This pathway would help leukemic cells survive treatment and eventually allow resistant BCR-ABL-T315I clones to grow out. BCR-ABL-activated SRC kinases alone may not transform B-lymphoid cells efficiently, but they are sufficient to maintain survival and stimulate proliferation of the leukemic cells. The next generation of BCR-ABL kinase inhibitors aims at increasing drug potency or overriding imatinib resistance caused by kinase domain point mutations, including BCR-ABL-T315I. However, to achieve a durable therapeutic effect in patients with Ph$^+$ B-ALL and lymphoid blast crisis, SRC kinases must be targeted. Our study suggests that targeting SRC kinases with dasatinib may delay transition of CML chronic phase to blast crisis and may be effective in treating acute lymphoid leukemia with compromised tumor suppressor function, providing a rationale for the early and continuous use of dasatinib in chronic-phase CML patients. The parallel results attained with the triple SRC kinase knockout cells and dasatinib demonstrate the role of certain pathways involving SRC kinases in the more advanced phases of CML and suggest that targeting SRC kinases and BCR-ABL with dasatinib may be an effective therapy for preventing transition of patients with chronic-phase CML to lymphoid blast crisis and for management of patients with advanced lymphoid leukemia. In fact, dasatinib is effective in treating Ph$^+$ B-ALL patients (24). If the BCR-ABL-T315I mutation is present in leukemic cells, this BCR-ABL-driven disease cannot be averted by dasatinib. However, dasatinib treatment may lead to long-term remission of B-ALL if the T315I mutation is absent from the leukemic cell population. The weak therapeutic effect of imatinib is unlikely to be attributed to an insufficient dose of imatinib, because the 100 mg/kg dose of imatinib administered in the mice inhibited BCR-ABL kinase activity *in vivo* significantly and to a similar degree compared with dasatinib.

Although dasatinib does not kill leukemic stem cells completely in B-ALL mice, targeting SRC kinases and perhaps other as-yet-unidentified signaling molecules could help achieve long-term control of the disease. Curative drug therapy of B-ALL would require targeting not only BCR-ABL kinase activity and SRC-dependent pathways, but also quiescent primitive leukemic cells (25). We identified pro-B leukemic cells as stem cells for B-ALL in mice. The rapid and striking hematologic response of B-ALL mice to dasatinib suggests that these pro-B progenitors with acquired self-renewal capacity are the major source of highly proliferating B-lymphoid leukemic cells in B-ALL mice and that complete inhibition of growth of this leukemic population could achieve long-term survival of B-ALL mice. Moreover, inhibiting the expansion of this population would reduce the frequency of the appearance of resistance mutations. It will be critical to assess whether BCR-ABL-expressing pro-B cells serve as stem cells

in patients with Ph^+ B-ALL or lymphoid blast crisis CML, because BCR-ABL can convert progenitors to leukemic stem cells (26). We also identified CML stem cells in mice as $Lin^-Sca\text{-}1^+c\text{-}Kit^+$ cells, and these cells are insensitive to inhibition by imatinib and dasatinib. Thus, identification of unknown pathways in CML stem cells will be critical for developing curative therapies for the disease.

Methods

Cell Lines. The BaF/3 pre-B and ENU cell lines were grown in RPMI medium 1640 containing 10% FCS, 10% WEHI medium, and 50 μM 2-mercaptoethanol.

Whitlock–Witte Culture. BM cells were transduced with the BCR-ABL retrovirus and cultured as described previously (5).

Antibodies and Western Blot Analysis. Antibodies against phosphotyrosine, c-ABL, CrkL, β-actin, and the SRC kinases were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); antibodies against c-Abl-Y412, phospho-CrkL, and SRC-Y416 were purchased from Cell Signaling Technology (Danvers, MA). Protein lysates were prepared by lysing cells in RIPA buffer, and immunoprecipitation and Western blotting were carried out as described previously (27).

BM Transduction/Transplantation. The retroviral vector MSCV-IRES-eGFP carrying the BCR-ABL cDNA was used to make virus stock as described previously (14). Four- to 10-week-old wild-type BABL/c or C57BL/6 (The Jackson Laboratory) and homozygous SRC triple gene knockout ($Lyn^{-/-}Hck^{-/-}Fgr^{-/-}$) mice (5) were used for leukemogenesis experiments (14, 28).

Flow Cytometry. Hematopoietic cells were collected from the diseased mice and analyzed by FACS analysis as described previously (5).

Drug Treatment. Dasatinib was dissolved in 80 mM citric acid (pH 2.1) to make 10 mg/ml stock solution and then diluted to 1 mg/ml with 80 mM citric acid (pH 3.1) for use. Imatinib was dissolved in water directly at a concentration of 10 mg/ml. The drugs were given orally in a volume of <0.5 ml by gavage twice a day, at 10 mg per kilogram of body weight per dose for dasatinib and 100 mg per kilogram of body weight for imatinib, beginning at 8 days after BM transplantation and continuing until the morbidity or death of the leukemic mice.

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1. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, *et al*. (2001) *N Engl J Med* 344:1031–1037.
2. Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, Holyoake TL (2002) *Blood* 99:319–325.
3. Marley SB, Deininger MW, Davidson RJ, Goldman JM, Gordon MY (2000) *Exp Hematol* 28:551–557.
4. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL (2001) *Science* 293:876–880.
5. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, Hallek M, Van Etten RA, Li S (2004) *Nat Genet* 36:453–461.
6. Wolff NC, Ilaria RL, Jr (2001) *Blood* 98:2808–2816.
7. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL (2004) *Science* 305:399–401.
8. O'Hare T, Pollock R, Stoffregen EP, Keats JA, Abdullah OM, Moseson EM, Rivera VM, Tang H, Metcalf CA, III, Bohacek RS, *et al*. (2004) *Blood* 104:2532–2539.
9. Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan-Jacob SW, Ray A, Huntly B, Fabbro D, Fendrich G, Hall-Meyers E, *et al*. (2005) *Cancer Cell* 7:129–141.
10. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M (2001) *N Engl J Med* 344:1038–1042.
11. Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, Talpaz M (2003) *Blood* 101:690–698.
12. Roumiantsev S, Shah NP, Gorre ME, Nicoll J, Brasher BB, Sawyers CL, Van Etten RA (2002) *Proc Natl Acad Sci USA* 99:10700–10705.
13. Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, Buchdunger E, Forster K, Moarefi I, Hallek M (2003) *Blood* 101:664–672.
14. Li S, Ilaria RL, Jr, Million RP, Daley GQ, Van Etten RA (1999) *J Exp Med* 189:1399–1412.
15. Wilson MB, Schreiner SJ, Choi HJ, Kamens J, Smithgall TE (2002) *Oncogene* 21:8075–8088.
16. Pear WS, Miller JP, Xu L, Pui JC, Soffer B, Quackenbush RC, Pendergast AM, Bronson R, Aster JC, Scott ML, *et al*. (1998) *Blood* 92:3780–3792.
17. Sill H, Goldman JM, Cross NC (1995) *Blood* 85:2013–2016.
18. Towatari M, Adachi K, Kato H, Saito H (1991) *Blood* 78:2178–2181.
19. Feinstein E, Cimino G, Gale RP, Alimena G, Berthier R, Kishi K, Goldman J, Zaccaria A, Berrebi A, Canaani E (1991) *Proc Natl Acad Sci USA* 88:6293–6297.
20. Williams RT, Roussel MF, Sherr CJ (2006) *Proc Natl Acad Sci USA* 103:6688–6693.
21. Holtz MS, Slovak ML, Zhang F, Sawyers CL, Forman SJ, Bhatia R (2002) *Blood* 99:3792–3800.
22. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC (1996) *J Exp Med* 183:1797–1806.
23. Heinrich MC, Blanke CD, Druker BJ, Corless CL (2002) *J Clin Oncol* 20:1692–16703.
24. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, Cortes J, O'Brien S, Nicaise C, Bleickardt E, *et al*. (2006) *N Engl J Med* 354:2531–2541.
25. Elrick LJ, Jorgensen HG, Mountford JC, Holyoake TL (2005) *Blood* 105:1862–1866.
26. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, Gotlib J, Li K, Manz MG, Keating A, *et al*. (2004) *N Engl J Med* 351:657–667.
27. Li S, Couvillon AD, Brasher BB, Van Etten RA (2001) *EMBO J* 20:6793–6804.
28. Roumiantsev S, de Aos IE, Varticovski L, Ilaria RL, Van Etten RA (2001) *Blood* 97:4–13.
29. Hunter T (1987) *Cell* 49:1–4.